Marking Juvenile Chum Salmon Otoliths by Immersion in Strontium Chloride and Calcein Solutions

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Mass marking of hatchery chum salmon has been identified as a critical need for effective management of large-scale salmon enhancement. Mass marking allows managers and researchers to assess the impacts of hatchery production on the environment, and on wild salmon stocks. Presently the accepted method of mass marking and identifying releases of chum salmon from large production hatcheries is otolith thermal marking. This technology is being employed by hatcheries in Alaska, Japan and Russia which together release nearly 1 billion otolith marked salmon yearly. However, the practical number of unique mark codes that can be applied is limited due to various factors of embryonic otolith development such as the physical size and the relatively short time available for inducing marks. The ability of readers to visually recognize marks is also limited. As more facilities utilize this technology, mark duplication will become more common. In addition, remote enhancement projects or studies of wild salmon stocks cannot usually implement this technology as it is not feasible to install and operate the heating or chilling equipment needed to induce otolith marks. The development of alternate techniques to mass mark chum salmon are needed, either as an adjunct to thermal marking or as a main marking tool for research on remote or wild stocks. Our project tests the efficacy of different immersion times or dosages of strontium chloride and calcein in order to develop and establish protocols for use in marking juvenile salmon.

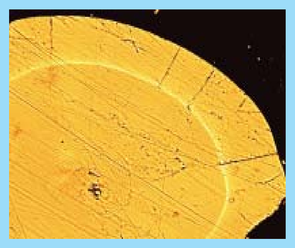
We used newly emergent chum salmon fry (approximately 32 mm fork length at emergence) from the DIPAC Ladd Macaulay hatchery in Juneau, Alaska for these marking trials.

The fry were immersion marked while still in fresh water. After marking they were reared in 31ppt seawater. Marking solution concentrations and immersion times are presented below:

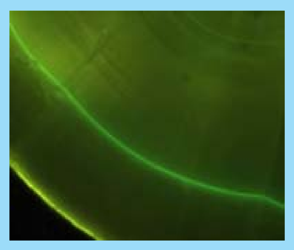
Strontium (g/l / h)	**Calcein** (g/l / m)
1/6 1/12 1/24 1/48	**1/1 1/3 1/5 1/10**
5/6 5/12 5/24 5/48	**5/1 5/3 5/5 5/10**
10/6 10/12 10/24 10/48	**10/1 10/3 10/5 10/10**

The fry to be marked with strontium were placed directly in the marking solution from fresh water. The fry which were to be calcein marked were placed in a weak salt solution for about 4 minutes prior to being immersed in the calcein solution. Each treatment was replicated three times. Three groups of unmarked fish were used as controls. The sample size for each replicate group was 150 fry. After marking (in mid-April), the fish from each treatment group were reared in small flow-through enclosures immersed in larger tanks (below). Samples of 10 fish were sacraficed from each treatment group at two week intervals through August, a period of about 15 weeks.

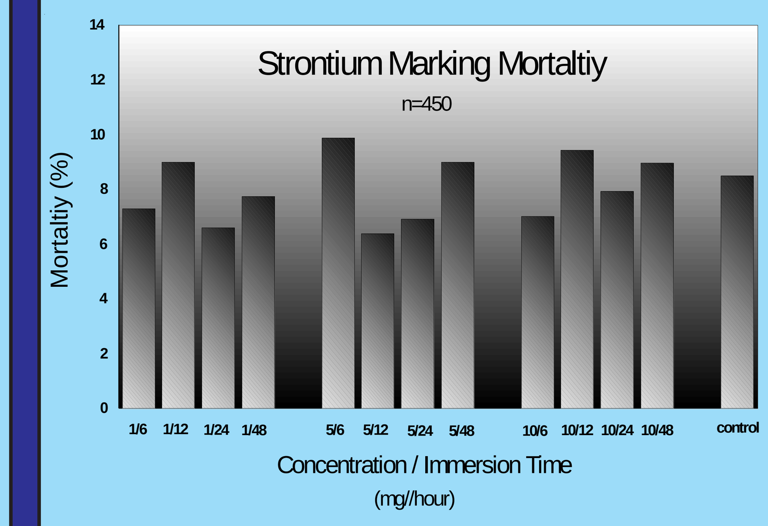
Right and left sagittae were removed from the salmon sampled one week and 8 weeks post marking (and also in week 15 for calcein marked fish). The otoliths were attached to glass slides with thermoplastic cement, ground to their medial plane, and polished. The strontium marked otoliths were further processed using a wave-length dispersive spectrometer to provide backscatter electron images of the otolith surface. The strontium mark appears as a bright yellow band. The otolith below left is unmarked while the otolith on the right is marked.

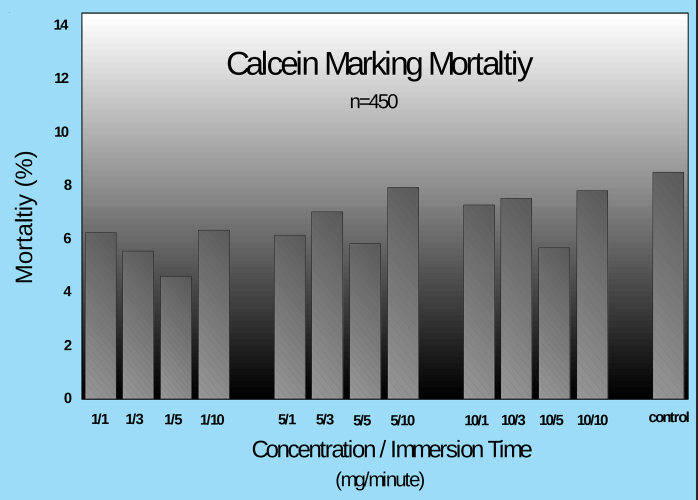


The calcein marked otoliths were examined using an ultraviolet light equipped compound microscope. The filter block recommended for viewing calcein incorporates a 450/490 bandpass filter, a 510 suppression filter, and a 515 longpass excitation filter. The calcein mark appears as a bright green band. The otolith below left is unmarked while the right otolith shows a strong calcein mark.



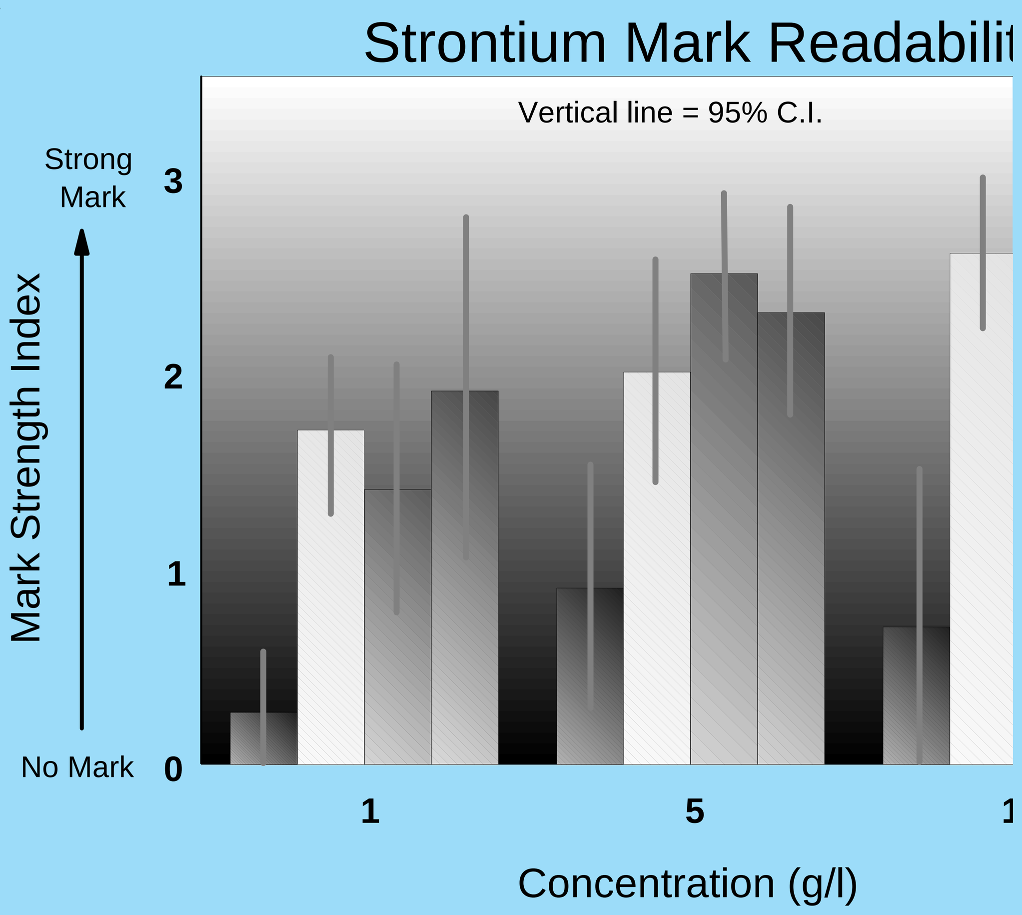
Solution concentration and immersion time in strontium chloride or calcein did not significantly affect the mortality of chum salmon fry over a 15 week time period. Mortality due to handling and marking in all treatment groups was similar and did not differ significantly from the mortality expressed in control groups (8.5%). Mortality in the strontium marked fish ranged from 7.1% (5g/l / 12h) to 8.9% (10g/l / 12h). Mortality in the calcein groups ranged from 6.2% (1g/l / 6m) to 7.3% (5g/l / 10m).



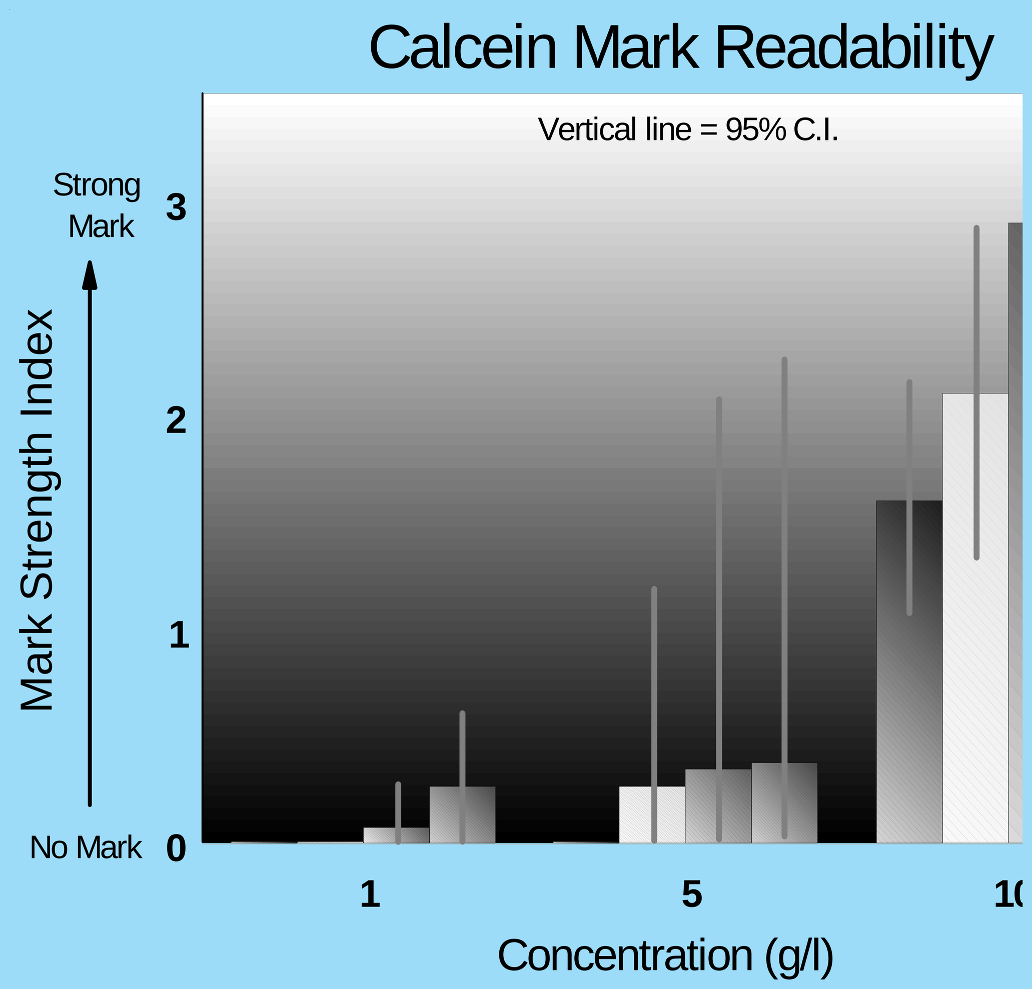


We examined the otolith images for marks. Mark readability was assessed using the following scale: 0 (no mark), 1 (doubtful mark), 2 (weak mark), 3 (strong mark). The otolith images were examined independently by three readers to provide a measure of mark readability. Immersion of the fish in either chemical produced readable otolith marks. However the expression of the respective chemicals within the otoliths were quite different and could vary widely by individual within the same treatment group. This was especially noticable in the calcein groups.

All strontium treatments produced marked otoliths. There was no significant difference between the 5/24, 5/48, 10/12, 10/24 and 10/48 groups and they all produced a quite readable otolith mark. The 1/6, 1/12, 1/24, 1/48, 5/6, 5/12, and 10/6 treatment groups produced doubtful to weak marks.



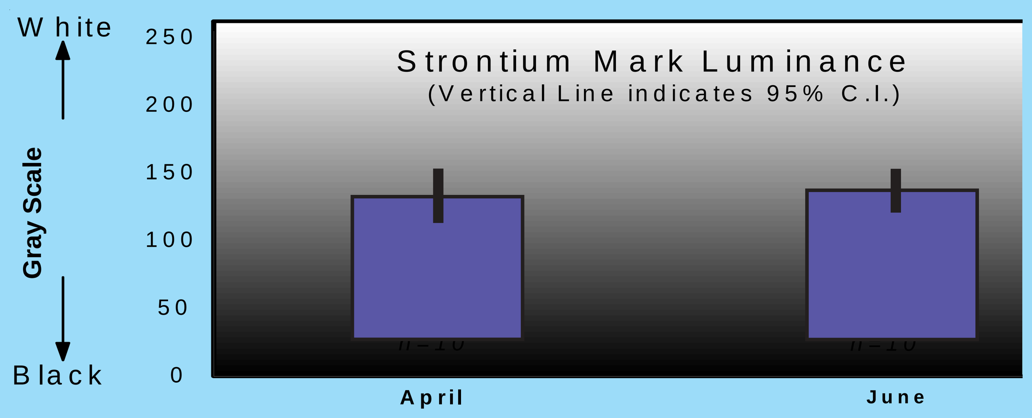
Several calcein treatments were unable to produce readable otolith marks (1/1, 1/3, and 5/1) while the 1/5, 1/10, 5/3, 5/5, 5/10 treatments produced very doubtful marks. The 10/1 and 10/3 groups were significantly more readable but still weak. The best results were produced by the 10/5 and 10/10 groups which consistantly produced strong marks.

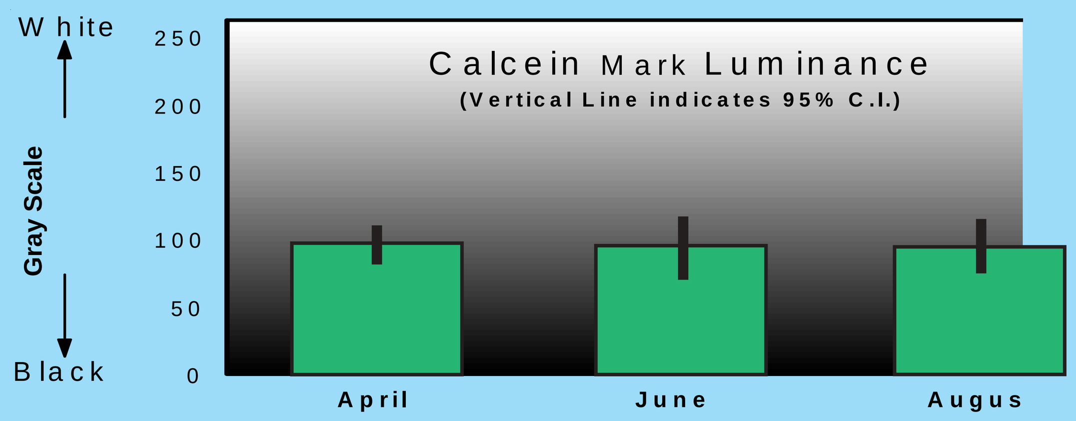


Next we wanted to determine if mark quality deteriorated over time. We had a serial collection of marked fish over a time period of about 15 weeks. We chose to examine fish one week after marking (mid-April), eight weeks after marking (mid-June) and (for calcein marked fish only) 15 weeks after marking (early August). Due to time and fiscal constraints we were unable to process the strontium marked fish from August.

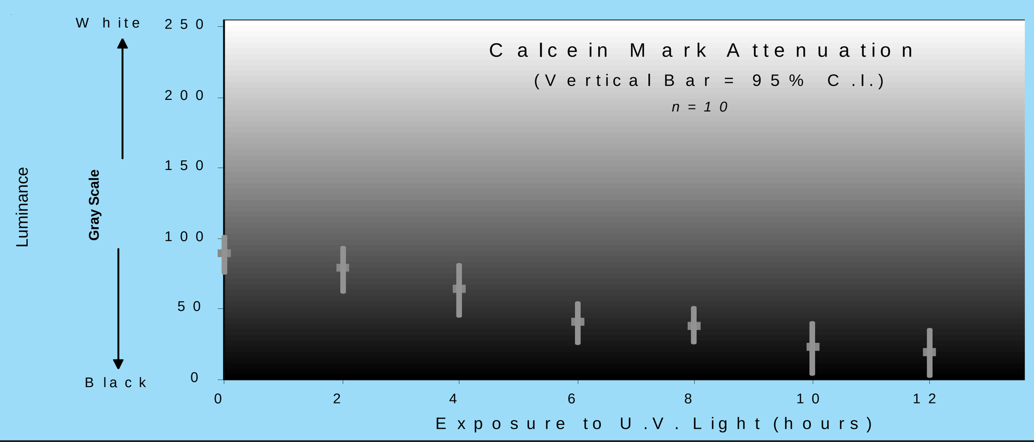
We examined otoliths from 10 fish in the same marking groups. The strontium marked fish came from 10/48 treatment groups while the calcein marked fish were from 10/10 groups. Using Optimas 6.5 digitizing program we determined the luminance of the otolith mark in the digitized image of each otolith. A decrease in mean luminance at successive sampling times would indicate a deterioration of mark strength.

We found that there was no significant decrease in mark luminance of either the strontium or calcein marked otoliths between April and June samples. In addition the luminance of the calcein marks did not decrease between June and August.





We were also interested in examining the effects that varying lengths of UV light exposure might have on the calcein marks. Some researchers expressed concern that lenghty exposure of the otolith to light (as can happen during processing) might cause a decrease in mark quality and readablity. Again we examined otoliths from 10 fish in the 10/10 calcein group. Using the Optimas 6.5 digitizing program we determined the luminance of the otolith mark in the digitized image of each otolith after mounting and grinding. Then measured again after 2, 4, 6, 8, 10, 12, and 14 hours exposure to intense incident UV light from the microscope. We found that the luminance of the calcein mark decreased significantly from an initial mean luminance of 92 to a luminance of 10 after 14 hours. After six hours of exposure the mark was about half of its original brilliance. This shows that exposure of calcein marked otoliths to light sources with substantial UV signatures during processing could lead to mark deterioration and errors in detection.



Conclusions:

Immersion marking chum salmon fry in strontium chloride and calcein did not result in increased mortality at the concentrations and immersion times tested.

Strontium chloride solution concentrations above 5g/l and immersion times of 12 hours and above produce readable otolith marks in chum salmon fry. These marks appear to be stable over time.

Calcein solutions of 10g/l and immersion times of 3 minutes or more also produce readable otolith marks in chum salmon fry. The calcein marks also appear to be stable over time unless the otolith is exposed to UV light. Our results show the calcein marks will become significantly less distinct within 6 hours of continuous exposure to a UV light source.

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